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Several transfer RNA genes of Sulfolobus solfataricus have been cloned and partially sequenced. Some of these clones are being used to determine the transcription initiation and termination sites of these genes and to study various post-transcriptional processing events.

The genes for some minor small RNAs, which migrate between 7S and 4S RNAs during polyacrylamide gel electrophoresis, have been cloned and are being characterized. In addition, facilities have been established to grow anaerobic thermophiles, e.g., Thermococcus celer, which is a strict anaerobe and grows optimally at 88°C and a pH of 5.5 to 5.8.

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ANNUAL REPORT

CONTRACT NO.: N00014-86-K-0739
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FULL TITLE: Structure and expression of various RNAs in the Archaebacteria.

ABBREVIATED TITLE: RNAs in Archaebacteria

INSTITUTION: The Board of Trustees of Southern Illinois University,
Carbondale, IL 62901

PRINCIPAL INVESTIGATOR: Ramesh Gupta
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PERIOD OF REPORT: September 1987 - August 1988

Summary of Project Goals:

1. Separation and sequencing of various RNAs of thermophilic archaebacteria.

2. Sequencing of various tRNA genes of archaebacteria (mainly thermophiles) and their surrounding regions to determine the organization of these genes and to identify the potential transcription control regions and the transcript processing sites.

3. Identification of transcription initiation and termination sites in tRNA genes of various thermophilic archaebacteria.

4. Characterization of RNA processing in thermophilic archaebacteria, initially using small transcripts, e.g., tRNA gene transcripts.

Recent Accomplishments

Several HindIII fragments of Sulfolobus solfataricus DNA containing tRNA genes have been cloned in pTZ18U and 19U vectors using standard techniques. Partial restriction maps for some of these clones have been generated. Specific regions of some of these clones have been partially sequenced. Some of these tRNA genes contain introns while others do not. The sequences of the genes of interest are being confirmed by sequencing the complementary strands of DNA. Several shortened plasmids have been generated during sequencing studies, by generating deletions in the inserts of the original clones. These various clones and their deleted versions will also be used during later transcriptional and RNA processing studies. Direct sequencing of different Sulfolobus tRNAs by various RNA sequencing methods appears to be difficult due to the presence of various 2'-0-methylated residues in these RNAs. Therefore, availability of the sequences of tRNA genes will be helpful in determining the tRNA sequences.

Studies are being conducted to determine whether intron containing tRNA genes are transcribed or not. Preliminary data indicate that at least some of these genes are transcribed in the cell, as their precursor transcripts are detected in the total RNA

preparations. These studies will be continued to study the mechanisms of excision of introns and splicing of exons in these transcripts. In addition, the sites for transcription initiation and termination of some of the tRNA genes are being determined.

We have observed several specific small RNAs, which are present in extremely small quantities in S. solfataricus. These RNAs migrate between 7S and 5S and between 5S and 4S RNAs during polyacrylamide gel electrophoresis. These RNAs do not seem to contain any modified nucleosides as observed by two-dimensional thin layer chromatography of RNAse T_2 digests of these RNAs. We are trying to determine whether these RNAs are precursors of some other stable RNAs or are products of RNA processing reactions. So far, we have not been able to directly sequence any one of these RNAs as it is difficult to isolate a specific RNA in large quantity and sufficient purity. Therefore, we are cloning the genes (DNA fragments) which hybridize to these RNAs. We have isolated some S. solfataricus clones which hybridize to these small RNAs. These clones are being characterized and sequenced.

Recently, we established the conditions to efficiently grow Thermococcus celer in serum bottles or other tightly capped bottles. The research machine shop at the University prepared a gassing manifold so that we can exchange the gases in closed systems (e.g., serum bottles) and grow anaerobic organisms. T. celer is a strict anaerobe, grows optimally at 88°C and a pH of 5.5 to 5.8. It also requires about 4% NaCl and elemental sulfur for its growth. It produces H_2S and some other strong smelling substances (probably mercaptans). Its growth ceases under high pressure of H_2S, which is immediately followed by lysis.

Plans for Next Year:

During next year, all the abovementioned works will be continued. Furthermore, since we can now grow T. celer, we shall also be using this organism to conduct studies similar to the ones mentioned before for S. solfataricus. Both of these organisms are thermophilic, yet they are phylogenetically sufficiently diverse to be classified into separate groups of archaebacteria.